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Camptothecin delivery systems: enhanced efficacy and tumor accumulation of camptothecin following its conjugation to polyethylene glycol via a glycine linker

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Abstract *Purpose*: This study was designed to assess the circulatory retention, antitumor activity and tissue biodistribution of polyethylene glycol (PEG)-conjugated camptothecin-20-*O*-glycinate, PEG-β-camptothecin (PEG-β-CPT). PEG-β-CPT is a novel water-soluble transport form (macromolecular prodrug) of the naturally derived antitumor drug, 20-(*S*)-camptothecin (CPT). *Methods*: Circulatory retention studies were performed in nontumor-bearing mice injected intravenously (i.v.) with 875 mg/kg of PEG-β-CPT. Antitumor activity was evaluated both intraperitoneally (i.p.) and i.v. in nude mouse xenograft models. Biodistribution studies were performed in nude mice bearing colorectal carcinoma xenografts with tritium-labelled PEG-β-CPT and CPT injected i.v. *Results*: PEG-β-CPT had a blood $t_{1/2\alpha}$ of approximately 6 min and a $t_{1/2\beta}$ of 10.2 h. Significant antitumor activity was seen in all treated xenograft models. Biodistribution studies demonstrated that PEG-β-CPT in saline provided more available labelled CPT in the circulation than unconjugated CPT dissolved in intralipid. In addition, it appeared that more labelled CPT accumulated in solid tumors when delivered in the PEG-β-CPT form, with greater preference for tumor tissue than normal tissue. *Conclusion*: This soluble transport form of CPT and its underlying technology may have clinical application especially for the treatment of solid tumors.

Key words Xenograft · Polyethylene glycol · Camptothecin · Prodrug · Biodistribution

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Introduction

Camptothecin (CPT), an extract from the chinese tree *Camptotheca acuminata*, has shown significant antitumor activity in nude mice bearing human lung, ovarian, breast, pancreas and stomach cancers [12]. Mechanistically, CPT stabilizes a topisomerase I-induced single-strand break in the phosphodiester backbone of DNA, preventing subsequent religation of the broken single strand. During replication, when the advancing fork encounters the covalent complex it results in a double-strand DNA break, which if left unrepaired, results in apoptosis. CPT, however, is extremely insoluble which has severely restricted its clinical application and has led many investigators to pursue water-soluble analogs [8, 20, 27]. However, a great deal of interest in pursuing water-insoluble CPT congeners still remains, because of their reported superior antitumor activity against in vitro human cancers and in vivo animal xenografts [24].

These two divergent issues can be resolved by employing a macromolecular prodrug strategy or, perhaps more accurately, a transport form [3, 13] of CPT. Prodrugs are temporary chemical modifications of the parent drug which are devised to enhance its aqueous solubility and biodistribution, while keeping its inherent pharmacological properties intact [23]. These transport forms are designed to be cleaved in vivo, in a predictable fashion, to the active drug by either an enzymatic mechanism or simple hydrolysis initiated under physiological pH conditions [31].

It has previously been reported that CPT can be solubilized as a nonionic α-alkoxyester conjugated to nonimmunogenic polyethylene glycol $(PEG)_{40kDa}$ [15]. Fortuitously, it has been found that modifying CPT at the 20 position as a PEG ester stabilizes the active lactone ring (essential for activity) under physiological conditions [15]. This 20-camptothecin PEG_{40kDa} ester (PEG-α-CPT) has been shown to hydrolyze in the blood gradually releasing the active ingredient CPT [15]. The solubility in water of CPT in the PEG-α-CPT transport

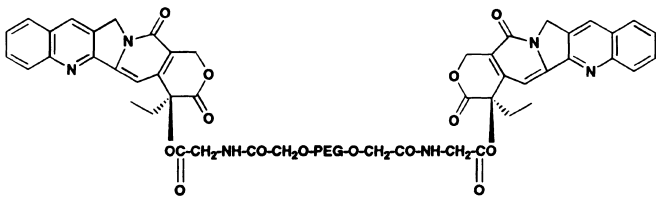

Fig. 1 Chemical structure of PEG-β-camptothecin (camptothecin-di-20-*O*-ester of PEG_{40kDa} glycine), a homologous soluble camptothecin disubstituted amidoester prodrug

form of minimally 2 mg/ml is dramatically greater than that of CPT (0.025 mg/ml) [20]. PEG-α-CPT exhibits a blood $t_{1/2\alpha}$ of approximately 4 min and a $t_{1/2\beta}$ of 3.5 h with significant antitumor activity [5]. However, PEG-α-CPT is a heterogeneous mixture of mono- and disubstituted ester prodrugs and therefore not clinically suitable. A new homogeneous form of disubstituted CPT has been achieved by employing a bifunctional spacer group (glycine) in the PEG prodrug strategy that yields a water-soluble nonionic α-amidoester prodrug, PEG-β-CPT (Fig. 1). In vitro P388/0 cell toxicity for PEG-β-CPT (IC_{50} 12 n*M*) is in the expected range for a prodrug that releases CPT (IC_{50} 7 n*M*) [16]. The in vitro half-life of hydrolysis of PEG-β-CPT to CPT at 37 °C is 40 h in pH 7.4 phosphate buffer and 6 h in rat plasma [16]. The objective of the current study was to assess in vivo circulatory retention, antitumor activity and tissue biodistribution of this new PEG-conjugated camptothecin-20-*O*-glycinate, PEG-β-CPT.

Materials and methods

Materials

PEG-β-CPT (camptothecin-di-20-*O*-ester of PEG_{40kDa} glycine) was produced as described previously [16]. Radiolabelled PEG-β-[^{3}H]CPT was prepared using 12-^{3}H-labelled CPT (Moravek Biochem. Brea, Calif.) and PEG_{40kDa} glycine acid synthesized according to a previously published procedure for PEG_{5kDa} glycine acid [14]. ^{3}H-CPT itself is expected to be relatively stable in biological systems [18]. Topotecan was synthesized according to published procedures [20]. For in vivo administration, 20-(*S*)-CPT was dispersed in intralipid (Liposyn III 10%, Abbott Laboratories, North Chicago, Ill.) by sonication. CPT was found to be stable in the intralipid vehicle as verified by high-performance liquid chromatography. 5-Fluorouracil (5-FU; Aldrich Chem. Co., Milwaukee, Wis.), formulated as previously prescribed [11], was used as a clinically relevant control for the xenograft experiments. All PEG-β-CPT dosages were dissolved in sterile water for injection (WFI) or physiological saline (0.9% NaCl) prior to in vivo drug treatments. With the exception of the circulatory retention study, all PEG-β-CPT dosages were given as their CPT equivalents (absolute amount of CPT given).

Circulatory retention

PEG-β-CPT circulatory retention studies were performed in 25 g nontumor-bearing CD1 female mice (Charles River Laboratories, Stone Ridge, N.Y.). Mice received an intravenous (i.v.) bolus of 875 mg/kg of PEG-β-CPT (14 mg/kg CPT equivalents) in saline via the tail vein and were exsanguinated over a 24-h period (0.05, 0.25, 2, 4, 6, 8 and 24 h) with three mice per time-point. Exsanguination was conducted in animals rendered unconscious in 100% CO_2 via orbital bleeding into a sterile tube to remove a minimum of 1.0 ml whole blood. Blood samples were processed and assayed as previously described [5]. The pharmacokinetics of injected PEG-β-CPT and released CPT were calculated using a two-compartment i.v. bolus first-order elimination model (WinNonlin, Scientific Consulting, Apex, N.C.).

Cell lines

HT-29 (human colon adenocarcinoma) was obtained from the ATCC (HTB38) and grown in DMEM supplemented with 10% fetal bovine serum (FBS). SKOV3 (human ovarian adenocarcinoma, ATCC/HTB77) was raised in McCoy's 5a medium supplemented with 15% FBS. A549 (human lung carcinoma, ATCC/CCL185) was grown in a 1:1 mixture of Dulbecco's modified Eagle's medium (MEM) and Ham's F-12, supplemented with 10% FBS. LS174T (human colon adenocarcinoma, ATCC/CL188) was cultivated in Eagle's MEM with nonessential amino acids and Earle's Balanced Salt Solution supplemented with FBS. All cultures were maintained at 37 °C in a humidified atmosphere of 5%CO_2/95%O_2 and subcultured once a week. All cell lines were periodically tested for mycoplasma and were mycoplasma free. Female nu/nu mice (Harlan Sprague Dawley, Madison, Wis.) at 18–24 g were inoculated subcutaneously into the left flank with tumor cells (1×10^6) in 0.1 ml medium.

Antitumor activity

Antitumor activity studies of PEG-β-CPT, given i.p. to HT-29 tumor-bearing nude mice, were conducted as previously reported [5]. Briefly, when tumors reached an average volume of 150 mm^3, the mice were divided into their experimental groups. Groups (n = 10) consisted of a control (untreated) group and groups treated with 5-FU, CPT, topotecan and a range of PEG-β-CPT dosages in WFI (1–4 mg/kg per day). Mice received CPT derivative treatments i.p. five times a week, Monday through Friday, for 5 weeks with 500 μl of test solution per treatment. Mice in the 5-FU treatment group were dosed (80 mg/kg) twice a week for 5 weeks. Mouse weight and tumor size were measured weekly over a 10-week period which spanned from tumor inoculation through post-treatment regrowth. The overall growth of tumors (%T/B) was calculated as the mean tumor volume at the end of the treatment minus the mean initial (pretreatment) tumor volume, divided by the initial tumor volume. Thus, any tumor group which did not respond to treatment and grew over the course of the experiment would display a positive percent change and treatment groups in which tumors regressed would exhibit a negative percent change.

Intravenous treatment with PEG-β-CPT against HT-29, SKOV3, A549 and LS174T were carried out in nude mice (seven or eight per group) bearing initial tumor volumes of approximately 110, 65, 100 and 65 mm^3, respectively. PEG-β-CPT in saline was given as a single dose (15 mg/kg CPT equivalent) or three doses (5 mg/kg CPT equivalent on each of days 1, 5 and 9) and tumor volume and body weight were measured weekly for 5 weeks. Tumor growth inhibition (%T/C) was used to determine antitumor effectiveness [6]. Treatment and control groups were measured when the control group's median tumor volume reached approximately 800–1100 mm^3 (exponential growth phase). The differences between treatment groups were assessed by one-way ANOVA. Multiple comparisons, when significant differences existed, were determined by least significant differences techniques. Statistical significance was defined as $P < 0.01$ to reject a null hypothesis. Statistical analysis was conducted using the StatView software program (Abacus Concepts, Berkeley, Calif.).

Biodistribution

Female nu/nu mice (Harlan Sprague Dawley, Madison, Wis.), bearing approximately 200 mm^3 HT-29 solid tumors were used. Xenografts were grown and animals maintained as described above. Each mouse received 16 mg/kg of either radiolabelled PEG-β-[^{3}H]CPT (in CPT equivalents) or [^{3}H]CPT with an activity of 100 μCi/kg. Anesthetized (0.9% Avertin, Aldrich Chemical Co., Milwaukee, Wis.) animals were injected i.v. via the tail vein using a 26-gauge needle. Treated mice were placed in metabolism cages throughout the experiment. At specified time-points postinjection, four mice per group were anesthetized, bled, sacrificed and their tissues removed. Samples were obtained at 0.8, 2, 6, 24, 48 and 72 h. Major organs (lung, heart, liver, spleen and kidney), muscle, tumor and blood were weighed, homogenized and counted via liquid scintillation in a Beckman LS 6000 IC counter (Fullerton, Calif.). Blood correction factors were applied to all organs [1, 10] and the data are expressed as the percent of the injected dose (%ID)/g tissue.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society of Medical Research and the "Guide for the Care and Use of Laboratory Animals" published by the National Institute of Health. These experimental protocols were approved by the Institutional Animal Care and Use Committee of UMDNJ-Robert Wood Johnson Medical School.

Results

Circulatory retention

A plot of the concentration versus time for PEG-β-CPT in mouse blood after an i.v. injection is shown in Fig. 2. The correlation between observed and predicted model time-point values was 99%. The blood $t_{1/2\alpha}$ was estimated to be 6 min with a $t_{1/2\beta}$ of 10.2 h, an area under the curve (AUC) of 53.3 mg/ml · h and a mean residence time (MRT) of 14.5 h. The inset in Fig. 2 shows the detected amount of CPT released in vivo from PEG-β-CPT over time. The AUC for released CPT was estimated to be 10 μg/ml · h with an estimated MRT of 18.9 h. The correlation between observed and predicted model time-point values was 99%.

Antitumor activity

The ability of multiple i.p. injections of PEG-β-CPT to affect the growth of HT-29 human colorectal xenografts was evaluated by monitoring tumor growth over a 5-week dosing period. All treatment groups were

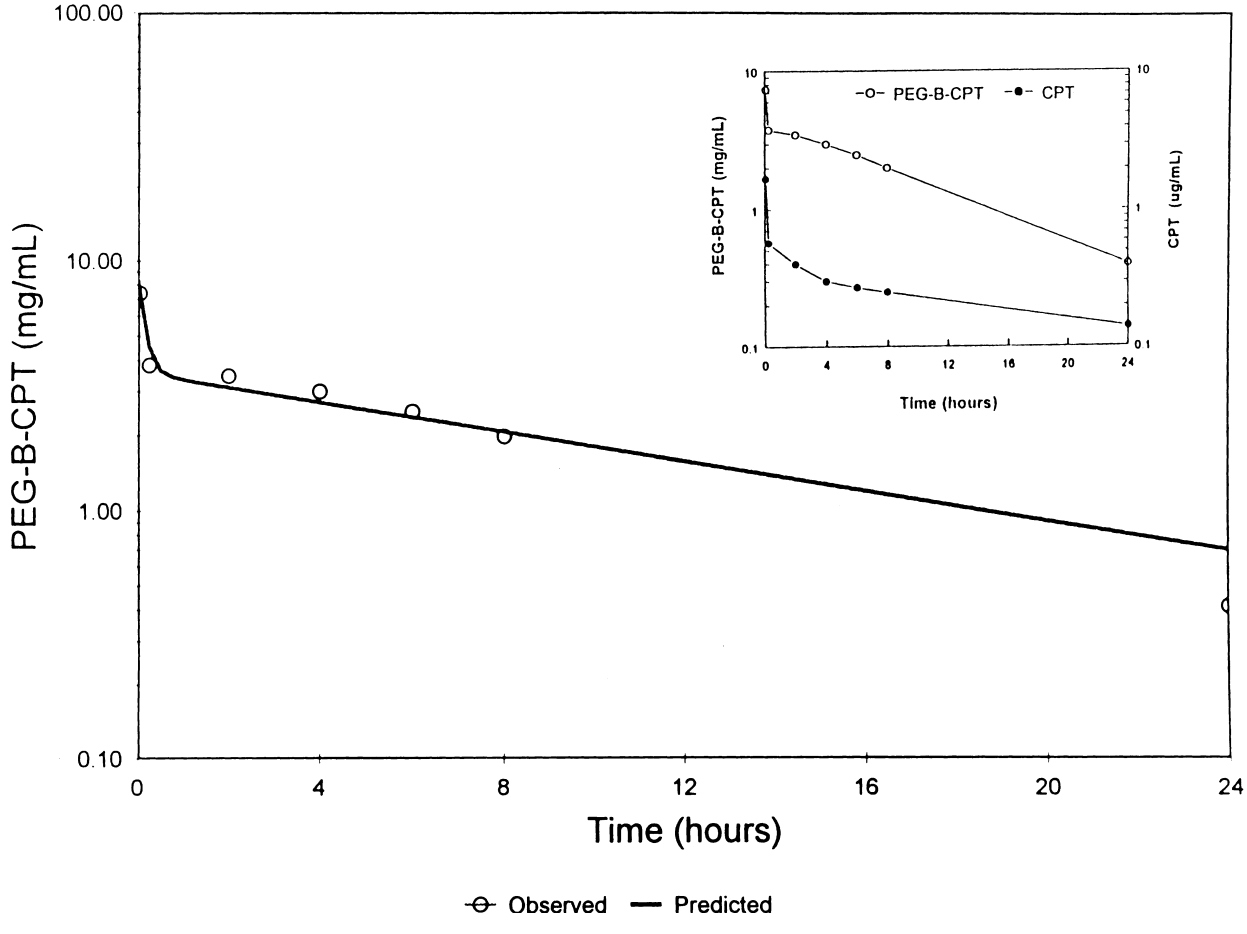

Fig. 2 Whole blood concentration-time curve of PEG-β-CPT (○) observed and model predicted (—) obtained after an 875 mg/kg i.v.-injection in mice. The predicted values were calculated using a two-compartment, intravenous bolus, first-order elimination model (WinNonlin, Scientific Consulting, Apex, N.C.) and displayed a 99% correlation with the observed values. *Inset graph* displays the detected amount of CPT released from PEG-β-CPT over time

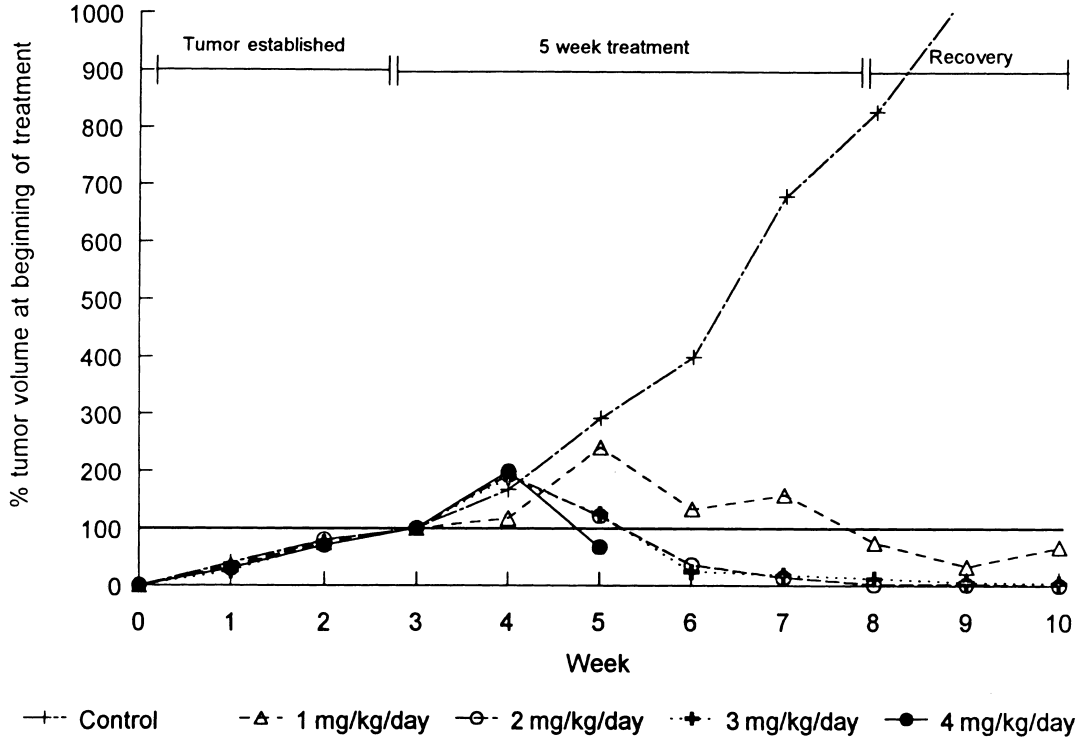

Fig. 3 Growth curve of HT-29 tumor xenografts treated with escalating dosages of PEG-β-CPT. PEG-β-CPT was given five times a week over a 5-week period. PEG-β-CPT dosages were based on CPT equivalents (absolute amount of CPT given). The initial mean tumor volume (mm^3) and SEM for each group ($n = 10$) were control, 150 ± 22; and PEG-β-CPT, 120 ± 22, 140 ± 16, 170 ± 19 and 90 ± 16 for the 1-, 2-, 3-, and 4-mg/kg dose groups, respectively

monitored for an additional 2 weeks in order to assess the short-term regrowth kinetics of the treated tumors. Figure 3 shows the effect of i.p. treatments with PEG-β-CPT on tumor volume. All doses of PEG-β-CPT caused tumor regression, with the 4 mg/kg dose regimen being toxic. CPT appeared more toxic (50% mortality) and less effective than equivalent doses of PEG-β-CPT (Table 1). Both 5-FU, which caused 30% mortality, and topotecan treatments resulted in tumor growth inhibition, but not tumor regression, during the 5 weeks of treatment (Fig. 4). Following drug treatment withdrawal, control, and 5-FU, CPT- and topotecan-treated mice all displayed tumor regrowth (Table 1). In contrast, the 2–3 mg/kg per day PEG-β-CPT dosage groups showed continued tumor regression, with the three groups displaying greater than 95% reduction in initial tumor volume.

Intravenous treatments with PEG-β-CPT in HT-29-, SKOV3-, A549- and LS174T-bearing mice demonstrated significant antitumor activity (Table 2). PEG-β-CPT was found to have similar antitumor activity when dosed either as a single high (maximum nonlethal) dose or a

Table 1 Antitumor activity against human colorectal tumor (HT-29) xenografts. In vivo efficacy study of the water-soluble CPT transport form, PEG-β-CPT, using the HT-29 human colorectal xenograft. A subcutaneous injection of HT-29 cells was allowed to reach an average tumor volume of 150 mm^3 prior to treatments

Treatment	Dose (mg/kg, i.p.)	Week 8 (end of treatment)		Week 10 (2 weeks post-treatment)		Total mortality
		Efficacy (%)[c]	Body weight change (%)	Efficacy (%)[c]	Body weight change (%)	
Control	–	727	+10	1047	+18	0/10
5-FU[a]	80	163	−8	341	+2	5/10
CPT	2.5	−20	−3	62	+9	5/10[d]
Topotecan	2.5	78	−8	300	+6	0/10
PEG-β-CPT[b]	1.0	−25	+1	−33	+12	0/10
	2.0	−98	−9	−100	+15	0/10
	2.5	−80	0	−96	+25	0/10
	3.0	−88	−11	−99	+19	1/10
	4.0	–	–	–	–	10/10[d]

[a] 5-FU given twice a week (Monday, Thursday) for 5 weeks
[b] Equivalent dose of camptothecin, mice dosed 5 days a week for 5 weeks
[c] Efficacy is expressed as % tumor volume change from initial (week 3)
[d] Toxic within 3 weeks of treatment

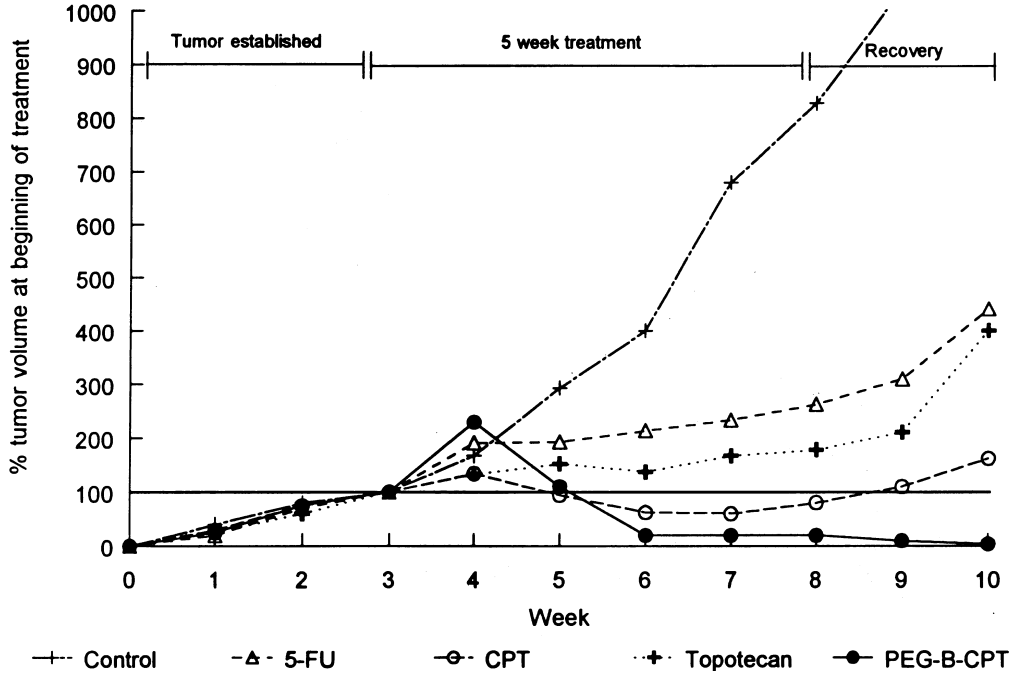

Fig. 4 Growth curve of HT-29 tumor xenografts treated with 5-FU, CPT, topotecan or PEG-β-CPT. All compounds were given over a 5-week period with CPT, topotecan and PEG-β-CPT being given five times a week (2.5 mg/kg per day) and 5-FU twice a week (80 mg/kg per day). PEG-β-CPT dosages were based on CPT equivalents (absolute amount of CPT given)

divided high dose given over a 9-day period (days 1, 5, 9). No significant weight loss or mortality was observed in PEG-β-CPT-treated mice.

Biodistribution

Table 3 shows the percent injected dose per gram tissue (%ID/g) for labelled CPT in both PEG-β-CPT and unconjugated CPT forms in tumor, blood, liver, kidney, spleen, lung, heart and muscle of nude mice bearing HT-29 tumors. Table 3 demonstrates the profound effect of PEG conjugation on the drug's biodistribution. Table 3 also shows that tumor tissue exposed to PEG-β-CPT demonstrated an increase in labelled CPT accumulation from 0 to 24 h, while unconjugated CPT had its maximal tumor concentration at 0.8 h and decreased thereafter. Figure 5 illustrates this increased tumor accumulation of labelled CPT when given in the PEG-β-CPT form as compared to CPT-injected mice. The tumor:tissue ratio (%ID/g of tumor divided by %ID/g of normal tissue) is shown in Table 4. The data indicate that from 2 to 72 h following PEG-β-CPT infusion, more radioactive material was detected in the tumor on a per gram basis than in liver, kidney, spleen, lung (except at 6 h), heart and muscle. This partitioning was not seen following unconjugated CPT administration.

Discussion

Both in vitro and in vivo data gathered with PEG-α-CPT and PEG-β-CPT suggest that the PEG transport forms release CPT in a relatively slow and sustained manner [5, 16]. This release in vivo is governed by the circulatory retention of the high molecular weight polymeric drug and its gradual dissociation. Thus, the rate of the conjugate's dissociation must be faster than

Table 2 Antitumor activity of i.v. administered PEG-β-CPT against subcutaneous human tumor xenografts in nude mice

Tumor type	Treatment schedule[a]	Total dose (mg/kg)[b]	%T/C[c]
HT-29 (colon)	Once daily × 1	15	9.5*
	Every 4 days × 3	16	14.3*
SKOV3 (ovarian)	Once daily × 1	15	25.2*
	Every 4 days × 3	16	21.4*
A549 (lung)	Every 4 days × 3	16	19.6
LS174T (colon)	Every 4 days × 3	16	14.3*

* $P < 0.01$
[a] Treatments initiated when tumor volumes reached 60–110 mm^3
[b] Based on CPT content
[c] Treatment and control groups were measured when the control group's median tumor volume reached approximately 800–1100 mm^3

Table 3 %ID/g of PEG-β-CPT and CPT at the times shown after i.v. injection in athymic mice bearing human colon adenocarcinoma xenografts. Each value is the average from four mice

Specimen	PEG-β-CPT						CPT					
	0.8 h	2 h	6 h	24 h	48 h	72 h	0.8 h	2 h	6 h	24 h	48 h	72 h
Tumor	0.47	3.34	3.34	3.70	2.35	1.63	0.33	0.11	0.11	0.10	0.05	0.05
Blood	27.90	19.17	10.91	4.41	1.94	0.73	1.21	0.27	0.08	0.09	0.07	0.07
Liver	1.62	2.02	2.00	2.32	1.89	0.79	4.32	0.54	0.29	0.14	0.07	0.07
Kidney	0.01	0.02	0.04	0.16	0.21	0.22	10.32	0.32	0.10	0.07	0.07	0.03
Spleen	0.01	0.03	0.03	0.07	0.31	0.49	2.73	0.15	0.26	0.14	0.03	0.03
Lung	3.68	2.33	4.00	1.95	0.24	0.33	3.09	0.64	0.19	0.12	0.04	0.05
Heart	2.13	1.87	1.74	1.13	0.88	0.41	1.32	0.17	0.12	0.09	0.09	0.03
Muscle	0.58	1.11	1.50	0.91	0.96	0.44	0.71	0.12	0.09	0.09	0.03	0.03

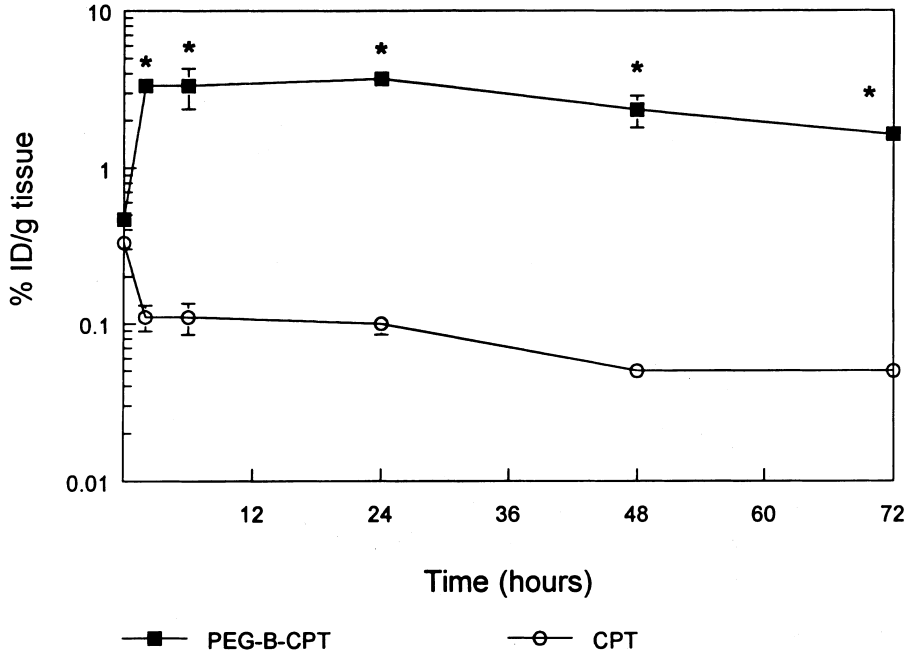

Fig. 5 Accumulation (mean ± SEM) of labeled CPT in the HT-29 tumors of nude mice (four mice/group per time-point) following a single i.v. injection of either PEG-B-[^{3}H]CPT or [^{3}H]CPT. *$P < 0.01$ vs CPT

its rate of circulatory elimination to allow sufficient emancipation of the bound drug. For this reason, the 40-kDa molecular weight polymer with its extended vascular retention [34] was employed as a carrier. The biodistribution experiment suggests that the long circulating life of PEG-β-CPT may be partly a consequence of reduced renal clearance, since high levels of unconjugated CPT were detected in the kidney at early time-points.

The PEG-β-CPT transport form demonstrated less toxicity and more efficacy than equal levels of unconjugated CPT in the i.p. HT-29 xenograft model. CPT with the 20-OH group modified is known to have greatly diminished activity [17, 19] and since the release of CPT

Table 4 Tumor:tissue ratio (%ID/g of tumor divided by %ID/g of normal tissue) of PEG-β-CPT and CPT at the times shown after i.v. injection in athymic mice bearing human colon adenocarcinoma xenografts. Each value is the average from four mice. Values greater than one indicate increased accumulation of labelled CPT in tumors over the corresponding tissue

Specimen	PEG-β-CPT						CPT					
	0.8 h	2 h	6 h	24 h	48 h	72 h	0.8 h	2 h	6 h	24 h	48 h	72 h
Blood	0.02	0.17	0.31	0.84	1.21	2.25	0.28	0.04	1.35	1.11	0.74	0.71
Liver	0.29	1.65	1.67	1.59	1.24	2.06	0.08	0.20	0.38	0.71	0.71	0.71
Kidney	47.0	167.0	83.5	23.13	11.19	7.41	0.03	0.34	1.10	1.43	0.71	1.67
Spleen	47.0	111.3	111.3	52.86	7.58	3.33	0.12	0.73	0.42	0.71	1.67	1.67
Lung	0.13	1.43	0.84	1.90	9.79	4.94	0.11	0.17	0.58	0.83	1.25	1.00
Heart	0.22	1.79	1.92	3.27	2.67	3.98	0.25	0.65	0.92	1.11	1.00	1.00
Muscle	0.81	3.01	2.23	4.07	2.45	3.70	0.46	0.92	1.22	1.11	1.67	1.67

from PEG-β-CPT is prolonged, the initial spiked release of cytotoxic CPT may be attenuated. Consequently, the lower level of freely circulating CPT may also reduce the amount of the potentially toxic [28] open carboxylate form. As far as gauging efficacy, free CPT was only able to reduce the HT-29 tumor burden by 20% over the 5 weeks of treatment, while the equivalent amount of CPT in the PEG-β-CPT form caused regression of the tumor size by 80%. This finding is not unreasonable, since a review of CPT studies [26] indicates that it is essential to maintain a low lactone plasma level, below the toxic threshold, over an extended period of time to achieve optimal therapeutic effects.

The biodistribution experiment demonstrated that conjugation augments the dissemination profile of ^{3}H-CPT in the mouse and results in the delivery of significantly greater amounts of radioactive material to solid tumors. PEG-β-CPT not only increased the total dose of CPT delivered to the tumor, but also provided higher tumor to tissue ratios over a longer period of time. In addition, the improved tumor to tissue ratios (i.e. from 2 to 24 h) of PEG-β-CPT were achieved with ^{3}H-CPT tumor levels at least ten times higher than those of unconjugated CPT. This enhanced tumor accumulation may be the basis for the divergent safety and efficacy effects seen between the conjugated and unconjugated forms of CPT in the xenograft model. In fact, a number of studies have shown that PEG-modified drugs cause increased intratumor accumulation together with an increased therapeutic index [9, 25, 29].

It is not surprising then, that, in addition to PEG-β-CPT's ability to solubilize and stabilize CPT [15] and thus change its inherent biodistribution, the transport form also has the innate characteristics of a passive tumor-targeting system. It is well established that macromolecules greater than roughly 50 kDa which circulate for extended periods, show substantial tumor-accumulation [21]. Indeed, PEG molecules of 10 kDa or greater molecular weight demonstrate a significantly higher accumulation in tumors than in normal tissue, irrespective of the tumor site [22]. One reason for this increased accumulation is the greater permeability of neovasculature in tumors [7, 33]. Another factor is the lack of an effective lymphatic drainage system in tumor tissue [30]. As a result, macromolecular drugs are retained in tumor interstitium for a prolonged period of time. The combination of increased tumor vascular permeability with insufficient tissue drainage results in what is termed the "enhanced permeability and retention effect", which is thought to be a universal solid tumor phenomenon for macromolecular drugs [21]. Although, [^{3}H]CPT in the PEG-β-CPT form demonstrated significantly greater tumor accumulation, which supports passive targeting of the entire moiety, intratumor build-up based on the circulatory release of CPT from PEG-β-CPT cannot be ruled out.

If passive targeting of the high molecular weight prodrug significantly contributes to CPT tumor accumulation, then PEG-β-CPT (a tripartate prodrug) [4] could exert its antitumor effects through two different cleavage mechanisms. The traditional ester hydrolysis seen with PEG-α-CPT (a bipartate prodrug) may occur which would liberate free CPT. An alternate pathway involving amide cleavage mediated by amino peptidases extracellularly could also occur leading to the small molecular prodrug, CPT-20-*O*-glycinate. This in turn could be taken up by the cell prior to breakdown [2], or since its $t_{1/2}$ is short in plasma [15], CPT may be generated simultaneously. Fortunately, since the tumor milieu is acidic [32], it is probable that the CPT lactone-carboxylate equilibrium is shifted in favor of the active lactone form, which could then enter the cell through either active or passive transport. Although, no significant difference in antitumor activity has been observed between the tripartate and bipartate PEG-CPT delivery systems [16], the dual pathways may become an important feature when treating other neoplasms or resistant cell lines.

In conclusion, conjugating PEG to CPT-20-*O*-glycinate results in a homogeneous water-soluble prodrug with an extended circulatory life and altered biodistribution, which generates greater tumor accumulation as compared to unconjugated CPT, thus producing significant antitumor activity. Work is now expanding on the effects of substituting other amino acids for glycine in the PEG-CPT delivery system, as well as examining different PEG-amino acid transport forms in a variety of in vivo applications. The data, to date, are convincing that this water-soluble transport form of 20-(*S*)-camptothecin, and its underlying technology, may have important clinical application.

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